Preparation and Evaluation of Efavirenz Loaded Silver Nanoparticles

Tanaya Balasaheb Pawar*, Mayur Prakash Sarode, Archana Rajkumar Dhole, Chandrakant Magdum

Rajarambapu College of Pharmacy, Kasegaon.

Rajarambapu College of Pharmacy, Kasegaon.

Rajarambapu College of Pharmacy, Kasegaon.

Rajarambapu College of Pharmacy, Kasegaon.

Phone No. 8806074915

Email ID: pawartanaya10897@gmail.com

Abstract:

Acquired Immuno Deficiency (AIDS) is the disease caused because of HIV which causes over 2 million deaths. HAART is plan that includes combination of drugs to inhibit infection of HIV. Many HIV infected patients are presently treated with the help HAART because of these HIV develops resistance for these drugs, requiring to alteration of medication regimen and resulting addition in expenditure of treatment. Application of nanoparticles to antiviral therapy development is possible. Nanoparticles are particles having minimum one dimension smaller than 100 nm. Antiviral agents act through interfering with infection of virus especially during the process of attachment and entry. Theoretically, some metal analyzed for activity against virus. Newly few studies have come forth to exhibit that metal nanoparticles can be used as antiviral agent against HIV-1.Silver nanoparticles suppress infection of HIV-1 through blocking entry of virus especially the CD4 and gp120 interaction.

Efavirenz is insoluble in water practically that causes poor bioavailability. Purpose of that study is to give additive effect as antiretroviral agent. Actuation of mechanism of Efavirenz is Non- Nucleotide Reverse Transcriptase Inhibitor (NNRTI) class of anti-retroviral agents. Many publication reported useful advantage of nanoparticles of silver to modify performance i.e. pharmacokinetic and pharmacodynamic, safety and targeted delivery of nanoparticles prepared by different methods like chemical, biological method, physical method. Efavirenz loaded silver nanoparticles can gives additive effect and rapid action. Alsofrom survey of literature it is observed that Efavirenz loaded nano sized particles of silver is best technique form management of AIDS as a antiretroviral agent.

Key Words:*Silver nanoparticles, Human Immunodeficiency Virus, Acquired Immunodeficiency Syndrome, Efavirenz, Silver nitrate.*

Introduction:

HIV means Human Immunodeficiency Virus which causes AIDS i.e Acquired Immunodeficiency Disorder. All around world it is considered as a major public health challenge globally. It is predicted that 36.7 million people in all worldwide are suffering from HIV. Average 1 million people died because HIV and causes related to it.(1)

In India HIV infection was first time detected in 1986 in Chennai in sex workers which is female. Now with an approximate 5.134 million people causes HIV/AIDS. India is home of second largest group of people having infection of HIV/ AIDS. (2) Global funds for TB, AIDS and Malaria,s executive director suggested that India pass South Africa in having greatest number of patient infected with HIV/AIDS in all around world in the year 2004. (3)

Approximately 50% people causes HIV/AIDS adults are female. In the country Africa women account for an approximately 76% people diagnosed with HIV between age of 15 to 24 years. (4)

The National Organization of AIDS Control in India estimated that by the completion of the year 2003 approximately 5.1 million people caused infection with HIV/AIDS. Maharashtra has highest prevalence in India and sex workers which is female has highest prevalence rate at 58.7%. In Mumbai marked growth in HIV casesbetween sex workers in female are observed because India is biggest sex industrial country. The prominent metropolitan city in Maharashtra, it increases from 10% in 1986 to 32 % in 1991 after increases to 54% in 2003. (5)

As per the National Organization for AIDS Control Latest Figure, in India there are 12 million of people cause infections of HIV. In India amount of patient infected with HIV/AIDS is continuously decreasing. Among that children are 6.54% and women are 40.5%. The national incidence of HIV dropped by approximately 0.38% in 2001, 0.34% in 2007, 0.28% in 2012 and 0.26% in 2015. (6)

In 2019 approximately 38 million patient infected with HIV/AIDS throughout the world among that 1.7 million are freshly infected and 6,90,000 people dies owing to HIV / AIDS related disorders. (7) As per current HIV estimate report of government, In India it is calculated that 23.49 lakh patient having infection with HIV/AIDS 2019. (8)

Virus of HIV causes infection and AIDS is a disorder in humans there is progressively immune system failure which allows life threatening infections. Infection of HIV occurs because of transfer of vaginal fluid, blood, semen, breast milk. In this body fluid HIV is found in the form of free virus particles or virus present in the infected immune cell. HIV causes infection to the vital cell of immune system like T cell, helper CD4 cell and macrophages. The human body has immune system having function to protect our body from the germs and infections etc. A person having infection with HIV doesn't have ability to fight against any disease. However immune system becomes poor. Virus is very small and simple which is inactive outside the human body and when it goes into the human body it becomes active. HIV is passed from infected individual to healthy individual and weakens immune system which causes deficiency of CD4+cell. White blood cells existing in immune system which helps in protection of body from several types of infections. A white blood cell contains CD4+ cells which is called as helper cells or T cells. HIV does not having ability to protect against disease so count of CD4 cells also decreases. (9)

Constituents of HIV:

GP41, GP120, Viral envelop, P17, P24, protease, integrase, RNA are HIV's constituents.

GP120: It is employed for attaching of virus to surface receptors of specific cell so it is require for entry of virus in cell.

GP41: It's a component of retroviruses envelop protein complex. By process of reverse transcriptase it is replicate within host cell. It is type of envelop virus.

Viral Envelop: It is helpful in the binding virus to cell.

P17: It is viral core which is made from protein having bullet like shape. There is need three enzymes for replication of HIV i. e. integrase, protease and reverse transcriptase.

P24: It is component of capsid of HIV.

Protease: It is essential for HIV lifecycle. Newly synthesized polyproteins are claves by enzyme protease at particular place to form protein components of HIV.

Integrase: It is the enzyme produced through retrovirus. It helpfull in integration of genetic material in infected cell DNA.

RNA: It is genetic material of retrovirus. (10)

Life Cycle of HIV:

There are 4 steps of HIV infection i.e. entry in the human cell, reverse transcription, transcription and translation, assembly, budding and maturation.

Entry of virus into human cell: HIV virus having ability to prepare new copies within the human cell. Virus entered in cell having protein cd4 on surface of cell. Virus attaches to cd4 receptor then fuse into it. HIV mainly causes infection to the immune cells like T helper cells so immune system of the body becomes weak.

Reverse Transcription: For this process of enzyme reverse transcriptase is required. It converts viral RNA into DNA. Then DNA transport into nucleus of cell. By the using enzyme integrase DNA insertion takes place.

Transcription and Translation: By using RNA polymerase enzyme make copy of genomic material of HIV and messenger RNA (mRNA). mRNA is useful in the preparation of long chain of HIV protein.

Assembly, budding and maturation: Copies ofmRNA comes together and make new HIV protein and enzymes and prepare new viral particles. By using enzyme protease long chain of HIV protein breakdown in smaller pieces and form new virus. This newly formed virus are ready to target and infect another CD4 cells. (11)

Efavirenz:

Drug Efavirenz is used in Highly Active Anti-retroviral Therapy for management of HIV-1. Brand name of Efavirenz is Sustiva and Stocrin. It having molecular formula $C_{14}H_9CIF_3NO_2$ and molecular weight is 315.68. Chemical name of efavirenz is (S)-6-chloro-(cyclopropylethynyl)-1,4-dihydro-4-(trifluromethyl)-2H-3,1-benzoxazin-2-one and its systematic IUPAC name is (4*S*)-6-chloro- 4-(2-cyclopropylethynyl)-4-(trifluoromethyl)-2,4- dihydro-1*H*-3,1-benzoxazin-2-one. It is a crystalline powder having colour white to slightly pink. It is practically not solublize in water having solubility < 10 µg/ml. Adult dose of drug efavirenz is 600 mg one time in a day. To minimize the psychiatric and neurological adverse effect it is taken at bed time in an empty stomach. Drug efavirenz having plasma half-life is ~50 hours. Efavirenz have some adverse effects also which includes psychiatric symptoms like nightmare, insomnia, confusion, depression, memory loss, psychosis occur in patient with cooperated kidney or liver function. According to general guidelines about pregnancy and drug efavirenz states that efaviernz having ability to because it has potential to cause birth abnormalities, it should not be used by pregnant women. (12)

Silver Nanoparticles:

Silver has interesting material characteristics and it is minimal cost natural resource. It also demonstrated to be effective against different variety of viral types involve HIV, herpes simplex,hepatitis B virus. Sosilver nanoparticles provide an intriguing resistance for drug. In conventional anti-viral treatment there are higher chances to create resistance for drug but in metal nanoparticles there is lowest possibility to produce resistance. The nanoparticles treatment for viral infection because they act through interfering in infection with virus mainly in attachment and entry of virus in cell. At small concentration silver is nontoxic to human beings. (13)

Antiviral agents interfering in attachment and entry in cell and suppress viral infection. Metal nanoparticles investigated for antimicrobial properties and have evidenced of antibacterial agent against gram negative and positive bacteria. Theoretically, few metals might be analyzed foractivity of antiviral, though slight effort done to define contact of viruses with metallic nanoparticles. Freshly few studies have developed presenting that metallic nanoparticles can be efficient antiviral agent against HIV-1.Silver nanoparticles inhibit initial stage of infection cycle of HIV-1 cycle by blocking adsorption and infectivity in a cell diffusion assay. It inhibits infection of HIV-1 through viral entry blocking especially the gp120 and CD4 interaction. (14)

Material and Methodology:

Efavirenz was obtained as a gift sample from Cipla, Goa. Silver nitrate, tri-sodium citrate and ethanol were obtained from Research Lab Fine Chem Industries, Islampur. All the chemicals and reagents used were of analytical grade.

Method of Preparation:

Silver NanoparticlesPreparation:

It is prepared by using chemical reduction method employing Turkevisch method. In this silver nitrate is utilized as precursor and tri-sodium citrate is utilized as reducing agent. For preparation of nano sized particles of silver take 500 ml water was taken and heat it at 80^0C to 100^0C. Add silver nitrate in this water. Dissolve tri-sodium citrate in another beaker containing 125ml water. Stir solution of $AgNO_3$ at 750 RPM. After 5minutes add tri-sodium citrate solution in it in drop wise manner then color changes from white to red and take it aside for whole night. Centrifuge this solution, then silver nanoparticles settled down. Wash this nanoparticles using ethanol. After that evaporate the ethanol and collect nanoparticles.(15)

Loading of Drug in SilverNanoparticles:

Prepare ethanolic solution of 100 mg drug and add 100 mg silver nanosized particles in this 5 ml ethanol and stir for 12 hrs. Then evaporate the ethanol and collect nanoparticles.(16)

Characterization and Evaluation:

1) Particle Size Analysis by Particle SizeAnalyser:

Using Particle size analyser (Horiba scientific SZ100), the vesicle size of efaviernz loaded silver nanoparticles were calculated. For particle size calculation sample was diluted using double distilled water. The particle size calculation depends on number of particles and ions that are present in solution.

2) Drug EntrapmentEfficiency:
After separating the silver nanoparticles by centrifugation, the supernatant solution was taken for quantification of drug in it. Accurately 1 ml solution from supernatant was diluted by using phosphate buffer and measured the absorbance to calculate unentrapped drug concentration. The entrapment efficiency of silver nanoparticles could be determined from formula which is given below.

Drug Entrapment Efficiency = Total Amount of Drug - Drug in Supernant Solution / Total Amount of Drug × 100

3) DrugRelease:
It is carried out by using dissolution type apparatus type 1 containing the basket. Precisely weighed quantity of Efavirenz silver nanoparticles (equivalent to 200 mg drug) was filled in hard gelatin capsules and put in basket, which was immersed in 900mlphosphatebufferhavingpH7.4.Thetemperatureofmediawaskeptat37°C ± 0.2 °C and stirred at speed of 75 rpm. Attime intervals of (1, 2, 3, 4, 5, 6, 7, 8 h) 1 ml samples were remove from bowl.Volume was substituted with same quantity of fresh warm dissolution medium. Filtered collected samples then analyzed at 247 nm, using UV-visible spectrophotometer against the phosphate buffer having pH 7.4 as a blank.(17)

4) Scanning ElectronMicroscopy:
Using a scanning electron microscope, the particle size of the optimized nanoparticles was measured and photographed.(16)

5) FT IRSpectroscopy:
It is utilized to interpret and determine functional groups in drug as well as excipients. FTIR of prepared silver nanoparticles was investigated by taking drug loaded silver nanoparticles directly on disk of ATR and continue for next phase in data monitoring which was display peaks on specific wavelengths. Following the creation of graphs peaks were discovered and the data were recorded. By using JASCO 4600, Japan spectrophotometer, the IR peak were studied. Data was interpreted using conventional values based on theresults. (18, 19)

Result and Discussion:

1) Determination of ParticleSize:
Fig 9.9 displays the particle size distribution of efavirenz loaded silver nanoparticles. The subsequent particle size study revealed maximum sensitivity in silver nanoparticles size distribution. The particle size of optimized batch was found that562.4 nm .

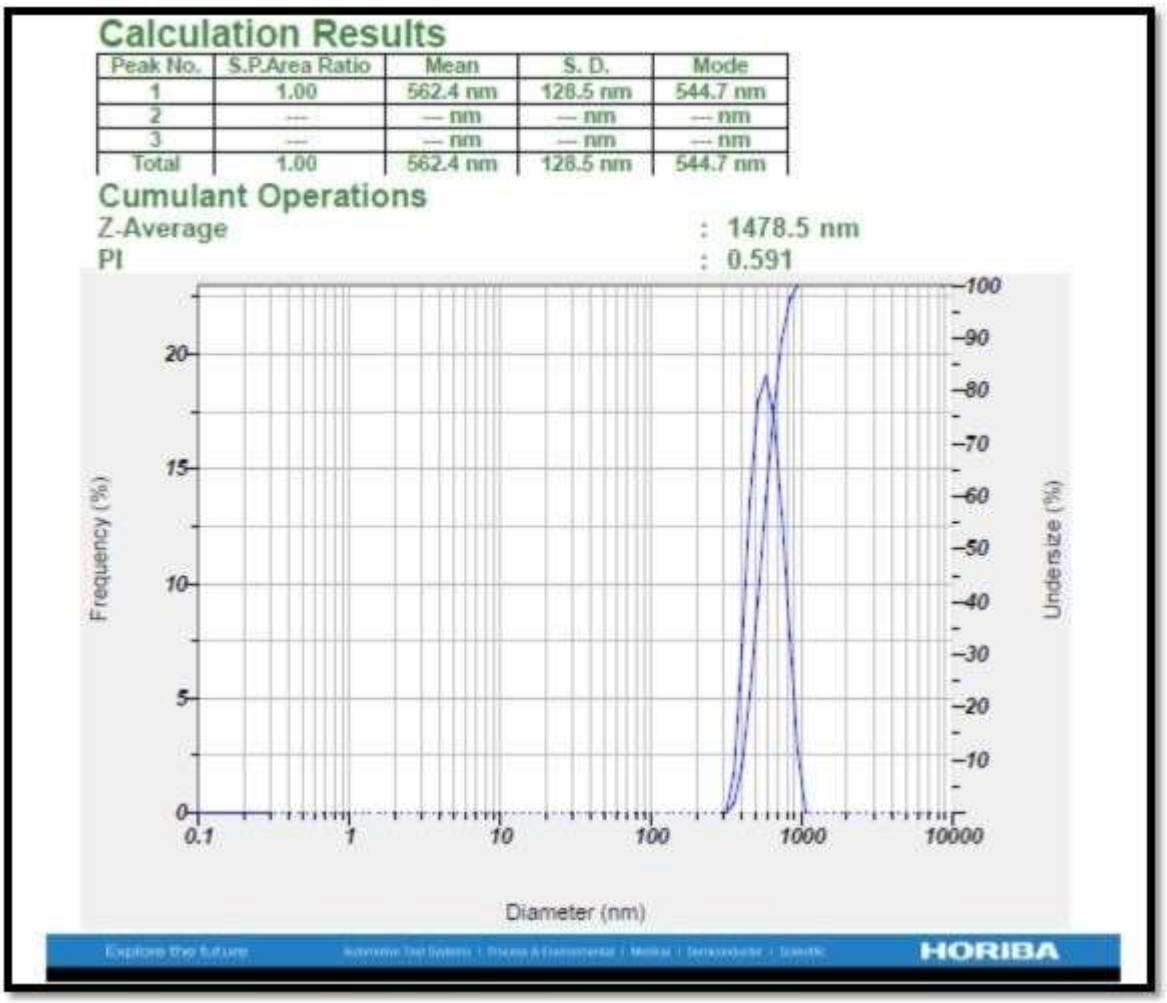


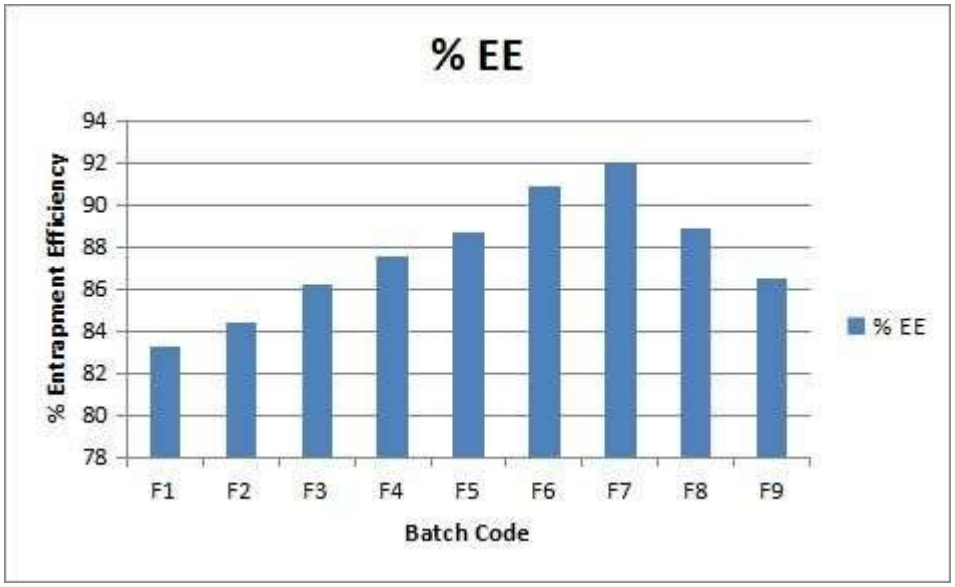
Fig No. 1 Particle Size of Optimized Batch

2) Drug EntrapmentEfficiency:
It was performed by ultracentrifugation method. Formulation No. 7 shows maximum entrapment efficiency i.e. 92%. Entrapment efficiency of formulation 1 to 9 was found that 83% to 92%. The result of study was summarized in table no.9.9

Table No. 1 Entrapment Efficiency from formulation 1 to 9

Sr. No.	Batch Code	Drug Entrapment Efficiency (%)
1	F1	83.3
2	F 2	84.4

3	F 3	86.2
4	F 4	87.6
5	F 5	88.7
6	F 6	90.9
7	F 7	92
8	F 8	88.9
9	F 9	86.5



Graph No.1 Shows % Entrapment Efficiency of efavirenz loaded silver nanoparticles from F1 to F9 Formulation

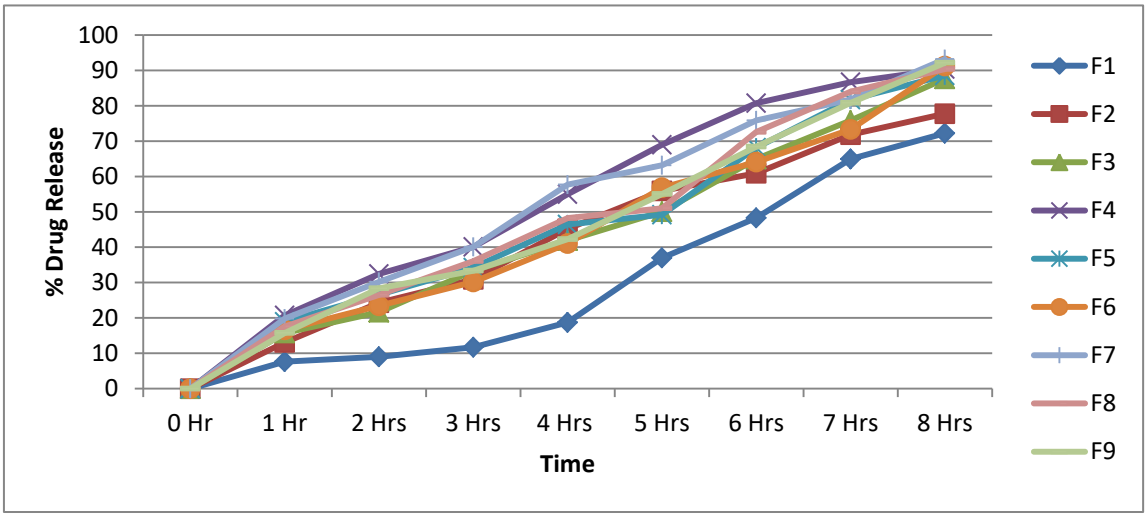
3) In Vitro DrugRelease:

It is conducted in PBS pH 7.4 by using dissolution testing apparatus. In present study, observed in vitro drug release for all F1 to F9 batches were in the series of 72.21 % to 93.20% displayed in table no.9.9 and fig 9.9. It was detected from the data that the in- vitro release of drug of silver nanoparticles formulation was performed for 8 hrs. Batch F7 shows higher % drug release . Graph shows increase in order of drug release while increase ontime

Table No.2 : Cumulative% Drug release of Efavirenz from Formulation 1 to 9

Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
0 Hr	0	0	0	0	0	0	0	0	0
1 Hr	7.65	13.05	15.75	20.7	18.9	16.65	19.8	17.55	15.75

2 Hrs	9	24.31	21.61	32.42	26.57	23.41	30.17	26.51	28.36
3 Hrs	11.7	30.19	33.34	40.10	34.25	30.19	40.10	36.04	33.34
4 Hrs	18.9	45.22	41.92	55	46.43	41.04	57.7	48.23	43.28
5 Hrs	36.95	55.92	50.05	68.95	49.19	56.82	63.16	50.98	55.03
6 Hrs	48.28	60.93	64.95	80.73	68.14	64.08	75.83	72.61	68.14
7 Hrs	64.94	71.80	75.85	86.67	81.72	73.30	81.76	84.0	80.82
8 Hrs	72.21	77.73	87.60	90.37	88.56	91.23	93.20	90.36	92.16



Graph No. 2 % Drug release of formulation F1 to F9

4) Scanning electron microscope:

The external morphology of Silver Nanoparticles surface as shown in Figure no. 9.10 indicated efavirenz loaded on the silver nanoparticles. (phenytoin)

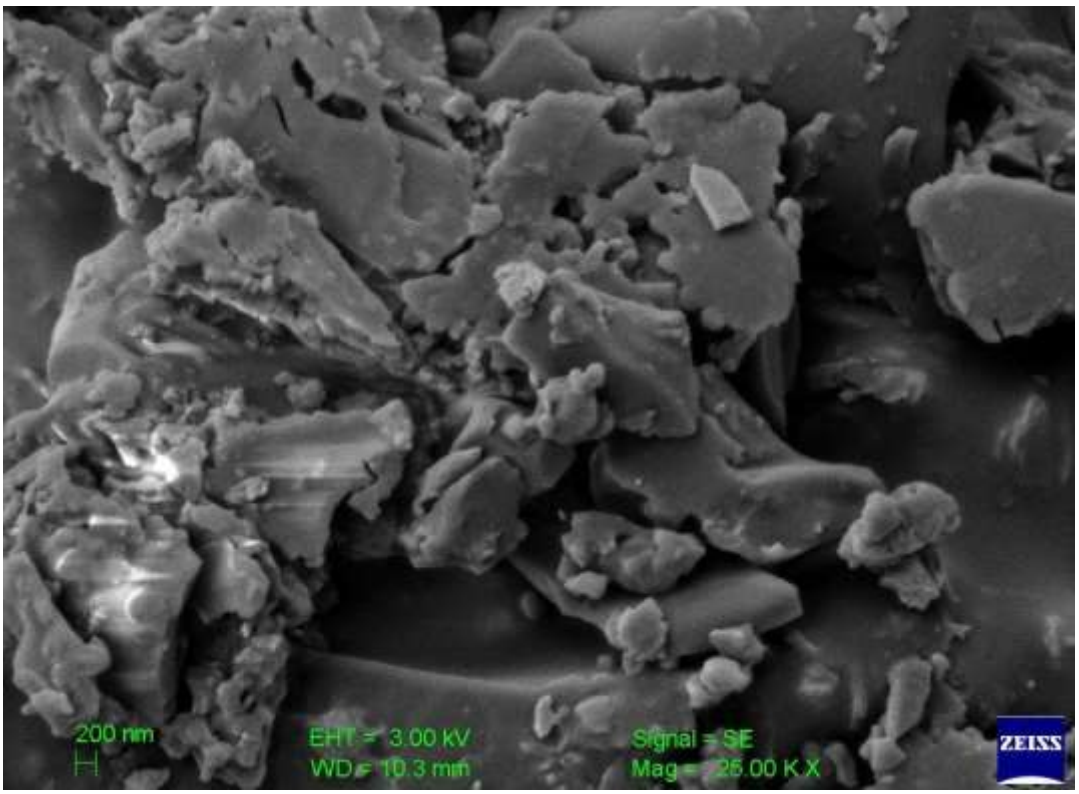


Fig No. 2. SEM image of silver nanoparticles.

5) FTIR of efavirenz loaded silver nanoparticles:

Fig No. 3 IR spectra of efavirenz loaded silver nanoparticles

Table No. 3 IR data interpretation of efavirenz loaded silvernanoparticles

Sr No.	Peak Position	Vibration	Functional Group
1	688.463	Stretching	C-Cl
2	808.028	Bending	C-H
3	1187.94	Stretching	C-O
4	1405.85	Stretching	C=C
5	1660.41	Stretching	C=O
6	2360.44	Stretching	C=C
7	3122.19	Stretching	O-H
8	3311.18	Stretching	N-H

Summary and Conclusion:

Based on preformulation studies drug and excipients were characterized for organoleptic characteristics, UV and FTIR spectroscopic study. The excipients and drug compatibility investigation were conducted. From obtained results drug and excipients discovered that it compatible to one another. There was not formation of any new compound.The drug loaded silver nanoparticle was prepared with the assistance of Turkevish method which is part of chemical reduction method. From obtained results of all silver nanoparticles batches it is concluded that optimum drug: excipient concentration is required.

The prepared silver nanoparticle batches were distinguished by several parameters like particle size, drug entrapment efficiency, % Drug releaseof prepared silver nanoparticles.

From different batches of silver nanoparticle formulation no. 7 shows maximal release of drug. The drug's release from prepared silver nanoparticles was done by using dissolution testing apparatus. The drug's release was found that93.23%. From this silver nanoparticles, batch F7 was optimized. The size of particle of batch F7 was discovered that562.4 nm.

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The optimized batch after evaluated for Scanning electron microscopy. Thedrug and excipients complex surface morphology was carried out by using Scanning electron microscopy.

In vitrorelease of drug, rate of release of drug was discovered that depends upon optimum drug, excipient ratio and particle size of nanoparticles. The formulation of silver nanoparticles of batch F7 show highest amount drug release from silver nanoparticles within 8hrs.

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From all result, formulation of silver nanoparticles batch F7 optimized formulation having % drug release and particle size 562.4 nm. An optimized formulation of silver nanoparticles of batch F7 was well acceptable and palatable with better absorption and stability

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